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(21) International Application Number: PCT/US99/15775 (22) International Filing Date: 13 July 1999 (13.07.99) (30) Priority Data: 60/092,650 13 July 1998 (13.07.98) US (71) Applicant: THE UNIVERSITY OF SOUTHERN CALIFORNIA [US/US]; University Park Campus, Los Angeles, CA 90089 (US). (72) Inventors: McKENNA, Charles, E.; 16625 Pequeno Place, Pacific Palisades, CA 90272 (US). LI, Zeng-Min; 1225 Benito Avenue #S, Alhambra, CA 91803 (US). LIU, Xue-Wei; - (US). (74) Agent: BERLINER, Robert; Fulbright & Jaworski L.L.P., 29th floor, 865 S. Figueroa Street, Los Angeles, CA 90017-2576 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: PREPARATION AND USE OF SULFUR-CONTAINING PHOSPHONOFORMATE DERIVATIVES AND ANALOGS (57) Abstract Preparation of phosphonoformic acid analogues containing one, two, three, or more sulfur atoms are described, with specific examples. Such compounds, not excluding cognates, derivatives, and homologues thereof, are proposed to be used directly, or as prodrugs, in treating viral infections, including but not limited to HIV, herpesviruses including HSV, EBV, VZV, CMV, HSV-6 and HSV-8 (Kaposi's sarcoma); HPV; rhinoviruses; and hepatitis-linked viruses. They are also proposed to be used in treating neoplasms, and for diagnosis and therapy of diseases of bone metabolism. The compounds of the present invention are also designed as to create novel biologically active compounds or prodrugs.		

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PREPARATION AND USE OF SULFUR-CONTAINING PHOSPHONOFORMATE
DERIVATIVES AND ANALOGS

CROSS-REFERENCE WITH RELATED APPLICATIONS

5 This application claims the benefit of U.S. Provisional Application No. 60/092,650,
filed July 13, 1998.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The invention relates generally to the field of phosphorus chemistry, and is
10 particularly concerned with a novel method for the production of sulfur-containing
phosphonoformates and their derivatives. An additional aspect of the present invention
relates to the use of these compounds as antiviral agents, including: Human
Immunodeficiency Virus (HIV); herpes viruses including, Herpes Simplex Virus (HSV),
Ebstein-Barr Virus (EBV), Varicella Zoster Virus (VZV), Cytomegalovirus (CMV), HSV-6,
15 and HSV-8 (Kaposi's sarcoma); Human Papilloma Virus (HPV); rhinoviruses; and hepatitis-
linked viruses.

DESCRIPTION OF RELATED ART

A retrovirus designated HIV is believed to be the causative agent of the complex
disease termed Acquired Immune Deficiency Syndrome (AIDS). The complex disease AIDS
20 includes progressive destruction of the immune system and degeneration of the central and
peripheral nervous system. The HIV virus appears to preferentially attack helper T-cells (T-
lymphocytes or OKT4-bearing T-cells) and also other human cells, e.g., certain cells within
the brain. The helper T-cells are invaded by the virus and the T-cells become an HIV virus
producer. The helper T-cells are quickly destroyed and their number in the human being is
25 depleted to such an extent that the body's B-cells as well as other T-cells normally stimulated

by the helper T-cells no longer function normally or produce sufficient lymphokines and antibodies to destroy the invading virus or other invading microbes.

While the HIV virus does not necessarily cause death per se, it does cause severe damage to the human immune system resulting in the onset of various other opportunistic diseases such as, herpes, toxoplasmosis, cytomegalovirus (CMV), Kaposi's sarcoma, and EBV related lymphomas, among others, (AIDS). HIV virus infected individuals, at first, experience few or no symptoms. Later in the disease, the onset of immune system dysfunction occurs, leading to various symptoms such as weight loss, malaise, fever, and swollen lymph nodes (AIDS related complex). The disease further progresses to full blown AIDS, leading usually to death. Those infected with the HIV virus are persistently infective to others.

Unfortunately, the present state of the art is such that antiviral drugs are only capable of attacking such viruses when they are replicating. Attacking a latent virus such as HIV, which does not reproduce itself following infection until reactivated by presently unknown factors would require distinguishing the viral genetic material from the surrounding host genetic material and selectively destroying it. Thus, the current generation of antiviral drugs is only effective against replicating viruses. Considerable efforts are being directed toward the control of HIV by means of inhibition of the reverse transcriptase of HIV and of other targets of viral functions, required for replication of the virus. Unfortunately, many of the known compounds suffer from toxicity problems, lack of bioavailability or are short-lived *in vivo*, viral resistance, or combinations thereof. Particularly important is combined therapy agents targeting more than one viral function. The requirement for development of new drugs able to be used in combination with other types of drugs is important in continued HIV therapy.

Several researchers have indicated that the pyrophosphonate analogues, such as phosphonoformic acid (PFA) and its analogues and derivatives possess antiviral properties in

- that they inhibit the replication of several viruses. (See, U.S. Patent Nos. 5,072,032 and 5,183,812 to McKenna; D.W. Hutchinson, et al., Synthesis and Biochemical Properties of Some Pyrophosphate Analogues, *Biophosphates and Their Analogues-Synthesis, Structure, Metabolism and Activity*, K.S. Bruzik and W.J. Stec (Eds.), Elsevier Science Publishers, B.V., 1987, 441-450; and Helgstrands, et al., *Science*, 201:819-821 (1978)). Trisodium phosphonoformate (PFA, Foscarnet), a pyrophosphate analog, has been reported to inhibit HIV reverse transcriptase (HIV, RT) with an ID₅₀ near 1 μ m, and has also been reported to inhibit several herpes virus DNA polymerases, including the DNA polymerase of CMV. See, U.S. Patent No. 5,072,032 to McKenna.
- Previous studies reported the addition of nucleophiles to the thiocarbonyl group. (See, J. Levillain, et al., *J. Am. Chem. Soc.* **115**, 8444-8446, 1993; and L.V. Kovalenko, et al., *Russian J. General Chemistry* 64, Part 1, 1456-1459, 1994). Preparations of thiocarbonyl and dithiocarboxyl derivatives have been demonstrated in the reaction of trialkyl phosphite and chlorothioformate. (See, D.W. Grisley, Jr., *J. Org. Chem.* **26**, 2544-2546, 1961.) However, trimethyl phosphonoformate thio derivatives have not been previously reported, except trimethyl thiophosphonoformate. U.S. Patent No. 5,072,032 to C.E. McKenna, et al., and trimethyl phosphonothionoformate, Kovalenko et al., *supra*. Corresponding monosodium salts and trisodium salts are disclosed for the first time, except for the monosodium and trisodium salts of thiophosphonoformate which were previously disclosed McKenna, et al, *supra*.

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BRIEF SUMMARY OF THE INVENTION

- The present invention solves the above-described problems by providing methods for readily synthesizing sulfur-containing phosphonoformic acid derivatives and the use of such derivatives as chemotherapeutic agents. In one embodiment of the invention, sulfur-
- containing phosphonoformate derivatives are obtained formally by replacing one or more of

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the five oxygen atoms of the original phosphonoformate molecule by a sulfur atom. Another embodiment of the present invention is a method for synthesizing polyhydroxy derivatives of such analogs. The method uses the base compound (foscarnet), but can be extended to prepare similar derivatives based on the thio analogues of foscarnet. An additional aspect of the present invention relates to the use of these compounds as antiviral agents, including: HIV and herpes viruses.

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following detailed description and appended claims.

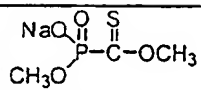
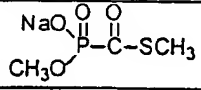
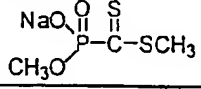
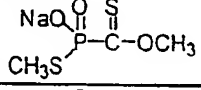
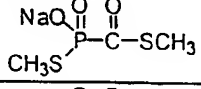
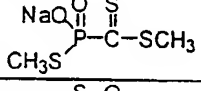
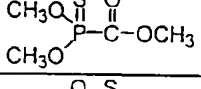
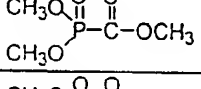
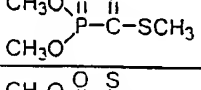
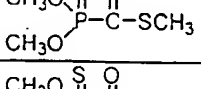
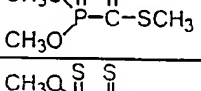
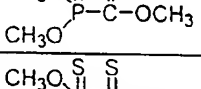
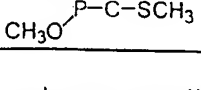
DETAILED DESCRIPTION OF THE INVENTION

The present invention teaches the synthesis of sulfur-containing phosphonoformic acid derivatives, which are obtained by replacing one or more of the five oxygen atoms of the original phosphonoformate molecule by a sulfur atom, see TABLE I. CAS is the Chemical

Abstract Service.

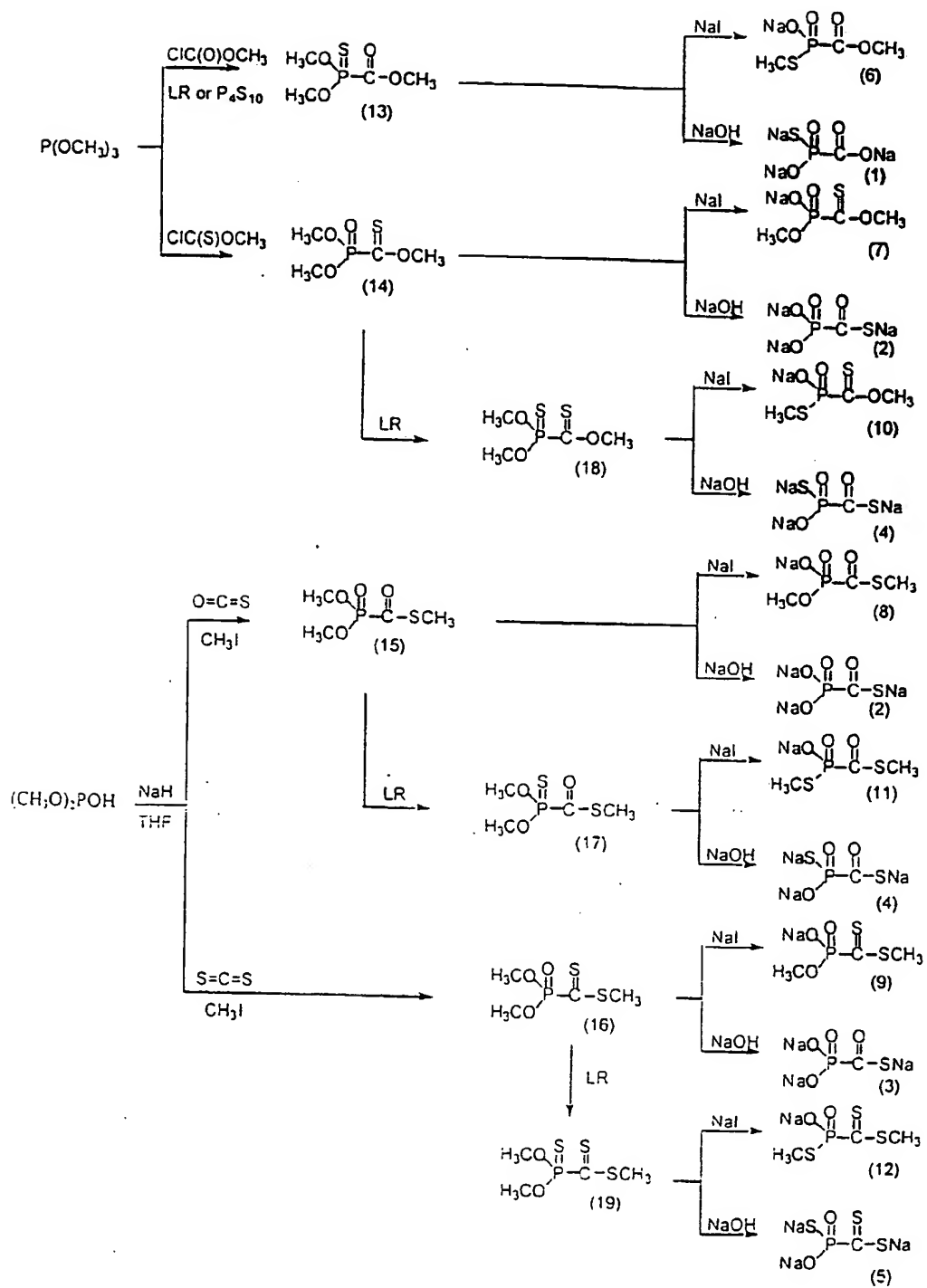
Table I. Selected Phosphonoformate Thio-Analogues

Name	Structure
Na ₃ TPFA, 1	$\begin{array}{c} \text{NaS} \quad \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ \text{NaO}-\text{P}-\text{C}-\text{ONa} \end{array}$
Na ₃ PTFA, 2	$\begin{array}{c} \text{NaO} \quad \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ \text{NaO}-\text{P}-\text{C}-\text{SNa} \end{array}$
Na ₃ PDTFA, 3	$\begin{array}{c} \text{NaO} \quad \text{O} \quad \text{S} \\ \parallel \quad \parallel \\ \text{NaO}-\text{P}-\text{C}-\text{SNa} \end{array}$
Na ₃ TPTFA, 4	$\begin{array}{c} \text{NaS} \quad \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ \text{NaO}-\text{P}-\text{C}-\text{SNa} \end{array}$
Na ₃ TPDTFA, 5	$\begin{array}{c} \text{NaS} \quad \text{O} \quad \text{S} \\ \parallel \quad \parallel \\ \text{NaO}-\text{P}-\text{C}-\text{SNa} \end{array}$
NaTPFA, 6	$\begin{array}{c} \text{NaO} \quad \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ \text{CH}_3\text{S}-\text{P}-\text{C}-\text{OCH}_3 \end{array}$

NaPTNFA, 7	
NaPTLFA, 8	
NaPDTFA, 9	
NaTPTNFA, 10	
NaTPTLFA, 11	
NaTPDTFA, 12	
Me3TPFA, 13	
Me3PTNFA, 14	
Me3PTLFA, 15	
Me3PDTFA, 16	
Me3TPTLFA, 17	
Me3TPTNFA, 18	
Me3TPDTFA, 19	

General synthetic pathways as applied to examples of various thio-analogs of PFA are outlined in the following conceptual schemes:

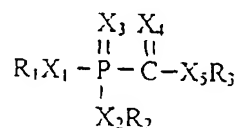
Scheme Table I



The unique biological activities of the compounds disclosed herein, and their corresponding classes, derive chiefly from two factors: 1) modification of their reactivity, cell transport, cell permeation, metabolism, and enzyme or membrane receptor site binding properties due to the different chemical and physical properties of sulfur relative to oxygen; and 2) potential *in situ* physiological conversion of S to O, creating the possibility of prodrugs for which the actual drug has one or more S converted to O after administration. Another factor is the modified properties of prodrugs or other analogues in which the sulfur-containing function is derivatized, e.g. as an ester, ether, etc., relative to metabolic activation *in vivo*.

THIO-ANALOGUES OF PFA

The analogues have the general structure:



R₁ and R₂ are each independently selected from alkyl, aryl, H, or cation. R₃ is independently selected from alkyl, aryl, H, or cation:

X₁, X₂, X₃, X₄, and X₅ are O or S, provided that:

(a) at least one of X₁ - X₅ is S;

(b) when X₁ is S, then either (i) R₁ or R₂ is alkyl, aryl, or H, or (ii) at least one of X₂, X₃, X₄, and X₅ is also S.

The parent structures may form part of a derived entity wherein R₁, R₂ and/or R₃ are more complex molecules than simple alkyl or aryl compounds (or portions of the same molecule), with the parent incorporated via one or more esteratic or ether bonds as indicated above. The cation can be a pharmaceutically acceptable alkali metal (e.g., Li, Na, or K), ammonium cation, alkaline earth cation (e.g., Ca²⁺, Ba²⁺, Mg²⁺), higher valency cation, or

- polycationic counter ion (e.g., a polyammonium cation). See. Berge, et al., "Pharmaceutical Salts", *J Pharm. Sci.* (1977) 66:1-19. It will be appreciated that the stoichiometry of an anionic compound to a salt-forming counterion (if any) will vary depending on the charge of the anionic portion of the compound (if any) will vary depending on the charge of the anionic
- 5 portion of the compound (if any) and the charge of the counterion. Preferred pharmaceutically acceptable salts include a sodium, potassium or calcium salt, but other salts are also contemplated within their pharmaceutically acceptable range. Furthermore R_1 , R_2 , and R_3 may be so designed as to create novel biologically active compounds or prodrugs, wherein one conjugating moiety may be for example a nucleoside or nucleotide with
- 10 independent activity, and another moiety may be for example a diol, triol or higher polyhydroxy group conferring enhanced cell transport or other desirable properties.

CLASSES OF PARENT STRUCTURES (X_1 - X_5 = O except as noted).

- The following compilation sets forth permutations for illustrative purposes. It will be appreciated that simple experiments by those skilled in the art will readily eliminate those
- 15 compounds that are not stable or are synthetically unattainable.

A. Triesters: R_1 , R_2 , R_3 = alkyl, aryl or complex

A.1: Monothio Analogues

$X_1 = S$	(A.1.a)
$X_2 = S$	(A.1.b)
$X_4 = S$	(A.1.c)
$X_5 = S$	(A.1.d)

A.2: Dithio Analogues

$X_1, X_2 = S$	(A.2.a)
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$X_1, X_3 = S$	(A.2.b)
$X_1, X_4 = S$	(A.2.c)
$X_1, X_5 = S$	(A.2.d)
$X_3, X_4 = S$	(A.2.e)
$X_3, X_5 = S$	(A.2.f)
$X_4, X_5 = S$	(A.2.g)

A.3: Trithio Analogues	
$X_1, X_2, X_3 = S$	(A.3.a)
$X_1, X_2, X_4 = S$	(A.3.b)
$X_1, X_2, X_5 = S$	(A.3.c)
$X_1, X_3, X_4 = S$	(A.3.d)
$X_1, X_3, X_5 = S$	(A.3.e)
$X_1, X_4, X_5 = S$	(A.3.f)
$X_3, X_4, X_5 = S$	(A.3.g)

A.4: Tetrathio Analogues	
$X_1, X_2, X_3, X_4 = S$	(A.4.a)
$X_1, X_2, X_3, X_5 = S$	(A.4.b)
$X_1, X_2, X_4, X_5 = S$	(A.4.c)
$X_1, X_3, X_4, X_5 = S$	(A.4.d)

A.5: Pentathio Analogues	
$X_1, X_2, X_3, X_4, X_5 = S$	(A.5.a)

Note: where $R_1 = R_2$, X_1 and X_2 are equivalent substitutions. However, compounds wherein

$R_1 = R_2$, such that X_1 and X_2 substitutions result in distinct isomers, are also within the

- 5 scope of the invention. Furthermore, stereoisomers, including stereoisomers created by the possibility of chirality at the phosphorus atom e.g. in a structural fragment like $M^+[R_1O(S)P(O)R_3]^-$ where M^+ is a cation are also within the scope of the invention.

B. Diesters

Note: where unspecified, R_1 , R_2 , and R_3 are H^+ or other cations.

B.1: Monothio Analogues	
<u>$R_1, R_2 = \text{alkyl/arvl/complex:}$</u>	
$X_1 = S$	(B.1.a)
$X_2 = S$	(B.1.b)
$X_4 = S$	(B.1.c)
$X_5 = S$	(B.1.d)
<u>$R_1, R_2 = \text{alkyl/arvl/complex:}$</u>	
$X_1 = S$	(B.1.e)
$X_3 = S$	(B.1.f)
$X_4 = S$	(B.1.g)

B.2: Dithio Analogues $R_1, R_3 = \text{alkyl/arvl/complex:}$ $X_1, X_2 = S$ (B.2.a) $X_1, X_4 = S$ (B.2.b) $X_1, X_5 = S$ (B.2.c) $X_2, X_3 = S$ (B.2.d) $X_2, X_4 = S$ (B.2.e) $X_2, X_5 = S$ (B.2.f) $X_4, X_5 = S$ (B.2.g) $R_1, R_2 = \text{alkyl/arvl/complex:}$ $X_1, X_2 = S$ (B.2.h) $X_1, X_3 = S$ (B.2.i) $X_1, X_4 = S$ (B.2.j) $X_3, X_4 = S$ (B.2.k)**B.3: Trithio Analogues** $R_1, R_3 = \text{alkyl/arvl/complex:}$ $X_1, X_2, X_4 = S$ (B.3.a) $X_1, X_2, X_5 = S$ (B.3.b) $X_2, X_3, X_4 = S$ (B.3.c) $X_2, X_3, X_5 = S$ (B.3.d)

$R_1, R_2 = \text{alkyl/arvl/complex}$:



B.4: Tetrathio Analogues

$R_1, R_3 = \text{alkyl/arvl/complex}$:



$R_1, R_2 = \text{alkyl/arvl/complex}$:



B.5: Pentathio Analogues

$R_1, R_3 = \text{alkyl/arvl/complex}$:



$R_1, R_2 = \text{alkyl/arvl/complex}$:



C. Monoesters:

C.1: Monothio Analogues	
<u>R₁ = alkyl/arvl/complex:</u>	
X ₁ = S	(C.1.a)
X ₂ = S	(C.1.b)
X ₄ = S	(C.1.c)
<u>R₃ = alkyl/arvl/complex:</u>	
X ₁ = S	(C.1.d)
X ₄ = S	(C.1.e)
X ₅ = S	(C.1.f)

C.2: Dithio Analogues	
<u>R₁ = alkyl/arvl/complex:</u>	
X ₁ , X ₂ = S	(C.2.a)
X ₁ , X ₄ = S	(C.2.b)
X ₁ , X ₅ = S	(C.2.c)
X ₂ , X ₄ = S	(C.2.d)
X ₄ , X ₅ = S	(C.2.e)

$R_3 = \text{alkyl/aryl/complex:}$

$X_1, X_2 = S$ (C.2.f)

$X_1, X_4 = S$ (C.2.g)

$X_1, X_5 = S$ (C.2.h)

$X_4, X_5 = S$ (C.2.i)

C.3: Tetrathio Analogues

$R_1 = \text{alkyl/ aryl/complex:}$

$X_1-X_4 = S$ (C.3.a)

$X_2-X_5 = S$ (C.3.b)

$X_1, X_3-X_5 = S$ (C.3.c)

$R_3 = \text{alkyl/ aryl/complex:}$

$X_1-X_4 = S$ (C.3.d)

$X_1-X_3, X_5 = S$ (C.3.e)

$X_1, X_3-X_5 = S$ (C.3.f)

C.4: Pentathio Analogues

$R_1 = \text{alkyl/ aryl/complex:}$

$X_1-X_5 = S$ (C.4.a)

$R_3 = \text{alkyl/ aryl/complex:}$

$X_1-X_5 = S$ (C.4.b)

D: Triacids and Salts:

D.1: Monothio Analogues	
$X_1 = S$	(D.1.a)
$X_4 = S$	(D.1.b)

D.2: Dithio Analogues	
$X_1, X_2 = S$	(D.2.a)
$X_1, X_4 = S; X_4, X_5 = S$	(D.2.b)

D.3: Trithio Analogues	
$X_1-X_3 = S$	(D.3.a)
$X_1, X_3, X_4 = S$	(D.3.b)
$X_1, X_4, X_5 = S$	(D.3.c)

D.4: Tetrathio Analogues	
$X_1-X_4 = S$	(D.4.a)
$X_1, X_3-X_5 = S$	(D.4.b)

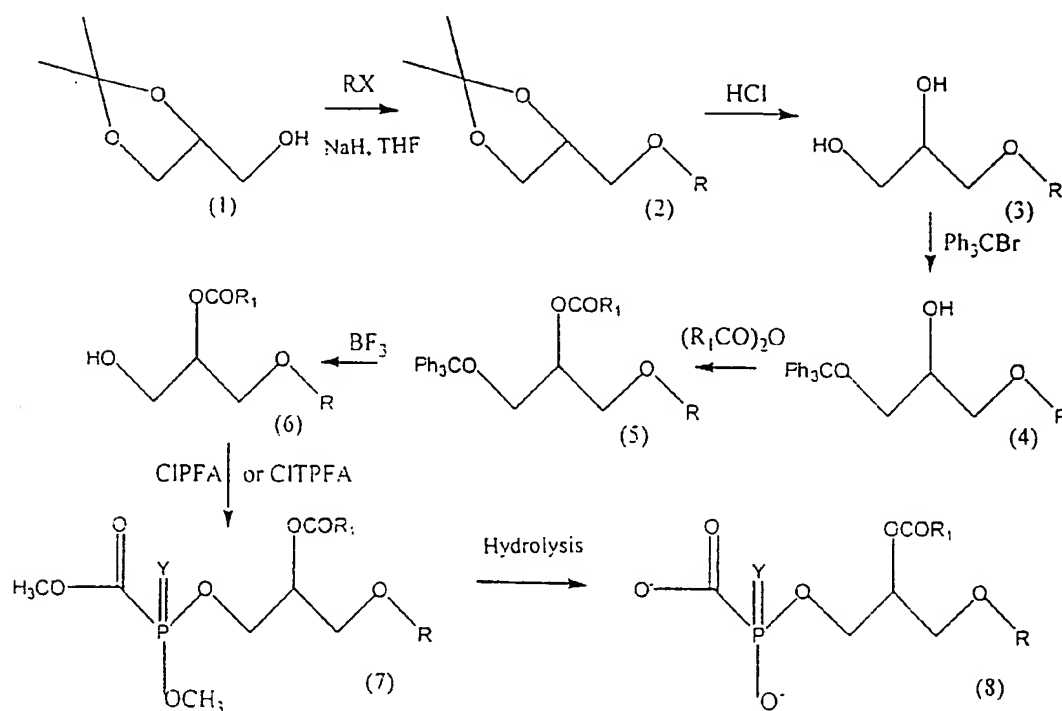
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D.5: Pentathio Analogues	
$X_1 - X_5 = S$	(C.5.h)

In accordance with this invention, TPFA is conjugated to polyalcohols. We postulate that such compounds can have enhanced membrane transport properties, and thus higher activity than the parent *in vivo*. Some 15 years ago, oral delivery of different classes of drugs was shown to be facilitated by incorporation of 1-O-alkyl, 1-O-acyl-sn-glycerol-phosphate moieties. See, Ryu, et al., *J. Med. Chem.* 25, 1322-1329 (1982). More recent examples of this approach have been given by M. Fuji, et al., *J. Org. Chem.*, 62, 6804 (1997) and by K. Hostetler, et al., *Antiviral Research*, 31, 59-67 (1996).

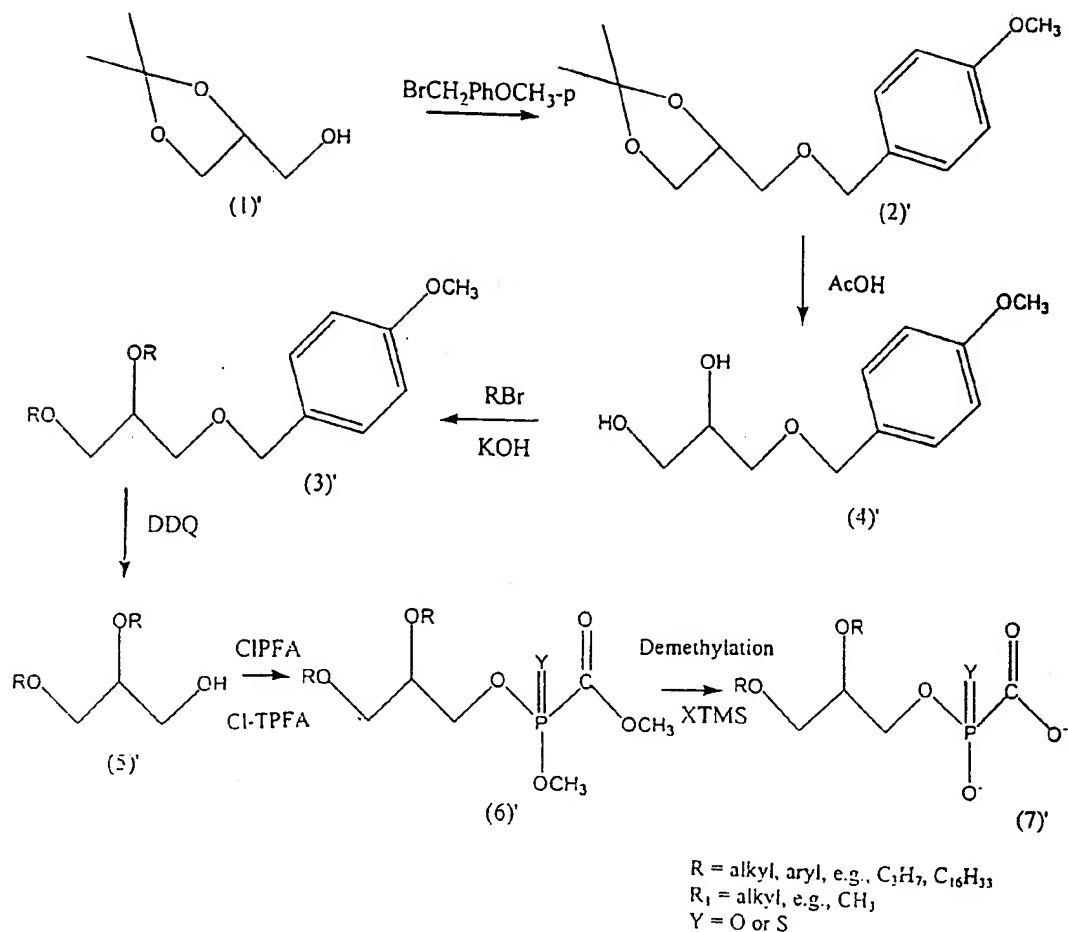
Our proposed synthetic routes are illustrated in the examples below.

Scheme II



R = alkyl, aryl, e.g., C_3H_7 , $C_{16}H_{33}$,
 R_1 = alkyl, e.g., CH_3 ,
 Y = O or S

Scheme III



Wherein R_1 is alkyl, such as C_3H_7 or $C_{16}H_{33}$; R_2 is alkyl, such as CH_3 ; and Y is O or S.

5

General Experimental Protocol

Solvents for reactions were purified as follows: THF was distilled from sodium and benzophenone ketyl, toluene was distilled from CaH_2 , and acetone was dried with molecular sieve. Solvents for column chromatography or Thin Layer Chromatography (TLC) were not pretreated. The end points of all reactions were checked using TLC or NMR except noted.

10 Monosodium salts and trisodium salts were dried in a vacuum, and all the products were stored at

4°C. R₁ is an alkyl group having 1 to 22 carbon atoms. R₂ is an alkyl group having 1 to 6 carbon atoms. X is a halogen, such as chlorine, bromine, and iodine.

All solvents and reagents were of Analytical Reagent (AR) grade quality, purchased from Sigma-Aldrich, Inc., without further purification except where noted. Nuclear Magnetic
5 Resonance (NMR) spectra were recorded on CDCl₃ solutions for triesters and on D₂O for monosodium salts and trisodium salts. ¹H and ¹³C spectra were recorded on Bruker AC250 MHz or AM360 MHz spectrometers, in CDCl₃. ¹H chemical shifts are referenced to CHCl₃ (δ 7.24). ¹³C chemical shifts were referenced to: CDCl₃ (δ 77.0); D₂O HDO (δ 4.63); and C₆D₆ (δ 128). ³¹P spectra were recorded on the 360 MHz instrument. ³¹P NMR chemical
10 shifts are referenced to external 85% H₃PO₄. Chemical shifts are reported in ppm (s = singlet, d = doublet, t=triplet, q = quartet, m = multiplet). High Resolution Mass Spectra (HRMS) determinations were performed at UC Riverside.

A. SYNTHETIC PROCEDURES FOR TRIMETHYL ESTERS

EXAMPLE 1

S-Methyl (Dimethylphosphinyl)thioformate

15 S-Methyl (Dimethylphosphinyl)thioformate, Table I Compound (15), was prepared by a previously published method (Kovalenko, et al., *Russian Journal of General Chemistry*, 64 (10) Part 1, 1456, (1994)). A solution of 1.00 g (9 mmol) of S-methyl chlorothioformate in 5 mL of
20 dry toluene was added over 10 min to a solution of 1.12 g (9 mmol) of trimethyl phosphite in 10 mL of dry toluene. The mixture was stirred and maintained below 30°C for 2 h. Toluene was removed by rotary evaporation under reduced pressure, and the resulting residue was distilled in vacuo, giving 0.72 g of a colorless oil, b.p.: 78-80°C/20 μ. The yield of product was about 55.6%.

EXAMPLE 2

S-Methyl (Dimethylphosphinyl)thioformate, Table I Compound (15), was prepared by a previously published method (Grisley, D.W., Jr., *J. Org. Chem.* 26:2544, (1961)). 11.0 g (0.1 mole) of dimethyl phosphite was added dropwise to a stirred solution of 2.4 g (0.1 mole) of sodium hydride in 50 mL of dry THF at 25-30°C under N₂. The mixture was stirred and refluxed until H₂ evolution ceased. The mixture was then cooled to 6°C and carbonyl sulfide gas was passed into the mixture until 6.7 g (0.11 mole) was absorbed. Methyl iodide (14.2 g, 0.1 mole) was then added at 5°C over 15 min. The mixture was poured into 350 ml of ice-water and extracted with ether (3 × 100 mL). The ether layers were combined, dried with anhydrous magnesium sulfate, filtered, evaporated to remove ether and distilled in vacuo. The fraction boiling between 87-89°C/100 μ was collected, 5.9 g, Y=32.1%. ¹H NMR: δ (ppm) 3.87 (d, 6H, ³J_{HP} = 11 Hz, POCH₃), 2.40 (d, 3H, ⁴J_{HP} = 1 Hz, CSCH₃); ¹³C NMR: δ (ppm) 197.7 (d, ¹J_{CP} = 208 Hz, C=O), 54.6 (d, ²J_{CP} = 7 Hz, POCH₃), 11.0 (d, ³J_{CP} = 4 Hz, CSCH₃); ³¹P NMR: δ (ppm) -1.44 (m, ³J_{PH} = 11 Hz); ³¹P NMR {H}: δ (ppm) -1.44 (s); HRMS: 185.0041 (MH⁺. Found), 185.0037 (MH⁺, Calcd).

- EXAMPLE 3

Methyl (Dimethoxyphosphinyl)thioformate, Table I Compound (14)

O-methyl chlorothioformate was prepared as follows: To a stirred solution of 40 g sodium hydroxide, 150 mL of water and 200 mL of methanol, cooled by means of an ice-bath, carbon disulfide (24 mL) was added dropwise over a 1 hour period. The mixture was treated with 0.1 g of KI and chlorine gas passed in until the purple color of free iodine was observed (end-point). The iodine color was discharged with 10% NaHCO₃. The mixture was washed with water (3 × 50 mL) and dried with CaCl₂. After filtration, a yellowish-green crude oil was obtained. ¹H

NMR: δ (ppm) 4.22. [See, Irwin B. Douglass and Glenn H. Warner. *J. Am. Chem. Soc.*, 78:6070 (1956)].

While cooled externally with cold water, 34 g of the crude oil described above was treated with 9 g of chlorine. The temperature should not exceed 30 °C to avoid over-chlorination. The mixture was heated slowly, then fractionated. The fraction boiling at 84-86°C/760 mm was collected, giving 12.7 g of O-methyl chlorothioformate, Y = 72%. ^1H NMR: δ (ppm) 4.16. [See, Dieter Martin and Wolfgang Mocke, *Chemische Berichte*, 98 (7), 2059 (1965).⁴] A solution of 0.55 g (5 mmol) of O-methyl chlorothioformate in 8 mL of dry toluene was added over 10 min to a solution of 0.62 g (5 mmol) trimethyl phosphite in 15 mL of dry toluene under N_2 . The mixture was stirred and left for 2 hr at room temperature. The solvent was rotary-evaporated, and the residue distilled in vacuo, giving 0.52 g of a yellow oil, 80-81°C /100 μ , Y = 56.5%. ^1H NMR: δ (ppm) 4.18 (d, 3H, $^4J_{\text{HP}} = 2$ Hz, COCH_3), 3.87 (d, 6H, $^3J_{\text{HP}} = 11$ Hz POCH_3); ^{13}C NMR: δ (ppm) 212.5 (d, $^1J_{\text{CP}} = 223$ Hz, PC), 59.1 (d, $^2J_{\text{CP}} = 8$ Hz, POCH_3), 54.8 (d, $^3J_{\text{CP}} = 6$ Hz, COCH_3); ^{31}P NMR {H}: δ (ppm) -1.14 (s); ^{31}P NMR: δ (ppm) -1.14 (m, $^3J_{\text{PH}} = 11$ Hz); HRMS: 185.0031 (MH $^+$, Found); 185.0037 (MH $^+$, Calcd).

EXAMPLE 4

Methyl (Dimethoxyphosphinyl)dithioformate, Table I Compound (16)

Dimethyl phosphite (11.0 g 0.1 mol) was added dropwise to a stirred solution of 2.4 g (0.1 mole) of sodium hydride in 50 mL of dry THF at 25-30°C under N_2 . The mixture was stirred under reflux until H_2 evolution ceased. It was then cooled to 6°C and was added with stirring to carbon disulfide (38 g, 0.5 mol) at 2-8°C over 15 min. Methyl iodide (14.2 g 0.1 mol) was then added at 5°C over 15 min. The mixture was quenched with 350 mL of ice-water and extracted

with ether (100 mL \times 3). The ether layers were combined, dried with anhydrous magnesium sulfate, filtered, evaporated to remove ether and distilled in vacuo. The fraction boiling between 92-94°C/50 μ was collected, 6.5 g, Y = 32.5%. (Put footnote 2 here) ^1H NMR: δ (ppm) 3.85 (d, 6H, $^3J_{\text{HP}} = 10$ Hz, POCH₃), 2.69 (d, 3H, $J_{\text{HP}} = 1$ Hz CSCH₃); ^{13}C NMR: δ (ppm) 228(d, $^1J_{\text{CP}} = 175$ 5 Hz, CS), 54.5 (d, $^2J_{\text{CP}} = 6$ Hz, POCH₃), 10.9 (s, CSCH₃); ^{31}P NMR {H}: δ (ppm) 0.30 (s); ^{31}P NMR: δ (ppm) 0.30 (m, $^3J_{\text{PH}} = 11$ Hz); HRMS: 199.9732 (Found); 199.9731 (Calcd).

EXAMPLE 5

The process of Example 4 was repeated for Methyl (Dimethoxythiophosphinyl)formate, 10 Table I Compound (13), with the following results: ^1H NMR: δ (ppm) 3.89 (s, 3H, COCH₃), 3.79 (d, 6H, $^3J_{\text{PH}} = 13$ Hz POCH₃); ^{13}C NMR: δ (ppm) 167.5 (d, $^1J_{\text{CP}} = 227$ Hz, CO), 54.5 (d, $^2J_{\text{CP}} = 6$ Hz, POCH₃), 52.9 (s, COCH₃); ^{31}P NMR { ^1H }: δ (ppm) 64.9. This is a compound previously described in McKenna's U.S. Patent Nos. 5,072,032 and 5,183,812.

15

EXAMPLE 6

The process of Example 4 was repeated for S-Methyl (Dimethoxythiophosphinyl)thioformate, Table I Compound (17), with the following results: ^1H NMR: δ (ppm) 3.85 (d, $^3J_{\text{HP}} = 12$ Hz, 6H, POCH₃), 2.39 (d, $^3J_{\text{HP}} = 12$ Hz, 3H, CSCH₃); ^{13}C NMR: δ (ppm) 198.4 (d, $^1J_{\text{CP}} = 163$ Hz, CS), 54.6 (d, $^2J_{\text{CP}} = 6$ Hz, POCH₃), 11.6 (s, CSCH₃); ^{31}P 20 NMR: δ (ppm) 68.0 ($^3J_{\text{PH}} = 12$ Hz); HRMS: 200.9827 (MH⁺, Found); 200.9809 (MH⁺, Calcd).

EXAMPLE 7

The process of Example 4 was repeated for Methyl

(Dimethoxythiophosphinyl)thioformate, Table I Compound (18), with the following results:

NMR: ^1H : δ (ppm) 4.19 (s, 3H, COCH_3), 3.87 (d, 6H, $^3J_{\text{PH}} = 11$ Hz POCH_3); ^{13}C NMR: δ (ppm)
 5 214.5 (d, $^1J_{\text{CP}} = 180$ Hz, CS), 59.5 (d, $^2J_{\text{CP}} = 9$ Hz, POCH_3), 55.0 (d, $J_{\text{CP}} = 7$ Hz, COCH_3); ^{31}P
 NMR (^1H): δ (ppm) 67.2; HRMS: 200.9815 (MH^+ , Found); 200.9809 (MH^+ , Calcd).

EXAMPLE 8

The process of Example 4 was repeated for Methyl

10 (Dimethoxythiophosphinyl)dithioformate, Table I Compound (19), with the following results: ^1H
 NMR: δ (ppm) 3.89 (d, 6H, $^3J_{\text{HP}} = 13$ Hz, POCH_3), 2.67 (d, 3H, $^4J_{\text{HP}} = 4$ Hz, CSCH_3), ^{13}C NMR:
 δ (ppm) 231.0 (d, $^1J_{\text{CP}} = 136$ Hz, CS), 55.0 (d, $^2J_{\text{CP}} = 7$ Hz, POCH_3), 19.8 (d, $^3J_{\text{CP}} = 3$ Hz, CSCH_3
); ^{31}P NMR (^1H): δ (ppm) 70.0; ^{31}P NMR: δ (ppm) 70.0 (m, $^3J_{\text{PH}} = 11$ Hz); HRMS: 216.9581
 (Found); 216.9581 (Calcd). Microanalysis: C%: 22.22 (Calcd), 22.24 (Found); H%: 4.19
 15 (Calcd), 4.25 (Found).

EXAMPLES 9 TO 11

General Procedure (using Lawesson's Reagent, LR)

LR (7.2 g, 17.8 mmol) was weighed out in a Dry Box and suspended in 100 mL of dry
 20 toluene. Trimethyl phosphonoformate, (11.4 mmol) in 10 mL of dry toluene was added. The
 mixture was refluxed for 6 h. The solid by-product was filtered, and toluene removed in vacuo.
 The residue was extracted with 40 mL of dry hexane, and the extract filtered, evaporated and
 distilled in vacuo to give the desired product.

EXAMPLE 9

The preceding general procedure was followed wherein the Me₃PTLFA was substituted for trimethyl phosphonoformate.

5

EXAMPLE 10

The preceding general procedure was followed wherein the Me₃PTNFA was substituted for trimethyl phosphonoformate.

EXAMPLE 11

10

The preceding general procedure was followed wherein the Me₃PDTFA was substituted for trimethyl phosphonoformate.

B. REACTION PROCEDURES FOR THE SYNTHESIS OF THE MONO-P ESTER
MONOSODIUM SALTS

15

EXAMPLES 12 to 16

General Procedure

In a 20 mL flask, 0.5 mmol of the corresponding trimethyl ester was dissolved in 15 mL of dry acetone. NaI (75 mg, 0.5 mmol) dissolved in 2 mL acetone was added, and the mixture was refluxed for 2 h. The mixture was then cooled, filtered, and the precipitate washed with dry
20 acetone until the wash was negative to AgNO₃. The final product was dried in a vacuum oven.

EXAMPLE 12

The preceding general procedure was followed wherein NaTPFA, Table I Compound (6), was synthesized. ¹H NMR: δ (ppm) 3.68 (d, 3H, ³J_{HP} = 1 Hz, POCH₃); 2.12 (d, 3 H, ³J_{HP} = 13
25 Hz, SCH₃); ¹³C NMR: δ (ppm) 171.0 (d, ¹J_{CP} = 150 Hz, CO), 50.81 (POCH₃); 10.43 (d, ²J_{CP} = 4

Hz. SCH₃); ³¹P NMR: δ (ppm) 18.1 (q, ³J_{PH} = 10 Hz); Microanalysis: C%: 18.76 (Calcd), 19.27 (Found); H%: 3.15 (Calcd), 3.06 (Found); S%: 16.69 (Calcd), 17.08 (Found).

EXAMPLE 13

5 The preceding general procedure was followed wherein NaPTLFA, Table I Compound (8), was synthesized. ¹H NMR: δ (ppm) 3.48 (d, 3H, ³J_{HP} = 11 Hz, POCH₃), 2.22 (s, 3H, CSCH₃); ¹³C NMR: δ (ppm) 207.4 (d, ¹J_{CP} = 190, CO); 53.3 (d, ²J_{CP} = 6 Hz, POCH₃); 10.1 (s, SCH₃); ³¹P NMR: δ -0.76 (q, ³J_{PH} = 11 Hz).

10

EXAMPLE 14

The preceding general procedure was followed wherein NaPTNFA, Table I Compound (7), was synthesized. ¹H NMR: δ (ppm) 4.01 (d, 3H, ⁴J_{HP} = 1 Hz, COCH₃), 3.47 (d, 3H, ³J_{HP} = 11 Hz, POCH₃); ¹³C: δ (ppm) 222.1 (d, ¹J_{CP} = 200 Hzs, CS); 58.9 (d, ²J_{CP} = 8 Hz, POCH₃); 51.8 (d, ³J_{CP} = 4 Hz OCH₃); ³¹P: δ (ppm) 0.26 (q, ³J_{PH} = 11 Hz).

15

EXAMPLE 15

The preceding general procedure was followed wherein NaPDTFA, Table I Compound (9), was synthesized. NMR: ¹H: δ (ppm) 3.46 (d, 3H, ³J_{HP} = 12 Hz, POCH₃), 2.52 (d, 3H, ⁴J_{HP} = 1 Hz, CSCH₃); ¹³C: δ (ppm) 239.0 (d, ¹J_{CP} = 150, CS); 53.3 (d, ²J_{CP} = 6 Hz, POCH₃); 18.9 (s, SCH₃); ³¹P: δ (ppm) 2.25 (q, ³J_{PH} = 11 Hz).

20

EXAMPLE 16

The preceding general procedure was followed wherein NaTPTLFA, Table I

Compound (11), was synthesized. NMR: ^1H : δ (ppm) 2.20 (s, 3H, CSCH_3), 2.05 (d, 3H, $^3J_{\text{HP}}$ = 12 Hz, PSCH_3); ^{13}C : δ (ppm) 204.2 (d, $^1J_{\text{CP}}$ = 140, CO); 12.0 (d, $^2J_{\text{CP}}$ = 4 Hz, PSCH_3); 10.5 (s, SCH_3); ^{31}P : δ (ppm) 22.2 (q, $^3J_{\text{PH}}$ = 12 Hz)..

C. PRODRUG SYNTHETIC APPROACH

EXAMPLE 17

Isopropylidene Glycerol, Scheme II Compound 1

Freshly distilled glycerol (10 g, 0.11 mole) was shaken until dissolved with 65 mL of dry acetone containing 1 g of TsOH . After 24 hr. the solution was neutralized first with PbCO_3 and afterwards with Ag_2CO_3 , warmed with charcoal, and shaken with a large excess of CaCl_2 overnight. The supernatant liquid was then filtered. The solvent was removed by rotary evaporation, and the product fractionated under diminished pressure, giving 9.5 g of the main fraction (b.p. $105\text{--}106^\circ\text{C}/25\text{mm}$) $\text{Y}=65.4\%$. NMR: ^1H : δ (ppm) 4.14-4.16 (m, 1H, CH), 3.63-4.0 (qxd, 2H, CH_2), 3.57 (m, 2H, CH_2OH), 2.05 (sb, 1H, OH), 1.40, 1.33 (CH_3); ^{13}C : δ (ppm) 109.4 (COO), 76.1 (CH), 65.7 (CH_2O), 62.8 (CH_2OH), 26.6, 25.2 (CH_3).

Propyl Isopropylideneglycerol Ether, Scheme II Compound 2

To a stirred solution of the protected glycerol (1.3 g, 10 mmol) in dry THF (20 mL) was added NaH (0.6 g, 25 mmol) portionwise at 0°C . After the reaction mixture was stirred for 30 min, 1-bromopropane (3.0 g, 25 mmol) was added. The mixture was stirred at r.t. Once the reaction was completed, 2 mL of methanol was added at 0°C to destroy the excess of NaH. The solvent was evaporated under vacuum. (b.p. $40\text{--}42^\circ\text{C}/50\mu$) The residue was

extracted with ethyl acetate. The extract was washed with water. After the dried solvent was removed, 0.86 g of a yellow oil obtained (Y=49%). NMR: ^1H : δ (ppm) 4.20-4.17 (m, 1H, CH), 3.65-4.0 (qxd, 2H, CH_2), 3.33-3.42 (m, 4H, CH_2OCH_2), 1.51 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 1.27, 1.33 (s, 6H, CH_3CCH_3), 0.82 (t, 3H, CH_3); ^{13}C : δ (ppm) 109.1 (COO), 74.6 (CHCH_2O), 73.2 (CH), 71.6 (CCH_2O), 66.7 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 25.2, 26.6 (CH_3CCH_3), 22.6 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 10.3 ($\text{CH}_2\text{CH}_2\text{CH}_3$).

Propyl Glyceryl Ether, Scheme II Compound 3

HCl (1 M, 2 mL) was added to a solution of 0.80 g (4.6 mmol) propyl isopropylidene ether in 3.0 mL of methanol and the reaction was stirred for 2 hr at room temperature. The reaction mixture was extracted with 3x 20 mL of ethyl ether, and the combined ether extracts were dried over sodium sulfate. After removal of the solvent, the residue weighed 0.51 g (Y=83.6%). NMR: ^1H : δ (ppm) 5.1 (m, 1H, CH), 3.37-3.68 (m, 6H, CH_2), 2.05 (sb, 2H, OH), 1.56 (2H, OCH_2CH_2), 0.88 (t, 3H, $\text{CH}_2\text{CH}_2\text{CH}_3$); ^{13}C : δ (ppm) 73.3(CH), 72.4 (CH_2OH), 70.5 (CH_2OCH_2), 64.2 (OCH_2CH_2), 62.7 (OCH_2CH_2), 10.4 (CH_3).

Benzyl Glyceryl Ether, Scheme II Compound 3, Alternative

As an alternative procedure to a stirred solution of the protected glycerol (6.7 g, 0.05 mole) in dry THF (100 mL) was added NaH (1.8 g, 0.075 mole) portionwise at 0°C. The reaction mixture was refluxed for 1.5 h, and benzyl bromide (10.3 g, 0.06 mole) was added. The mixture was stirred at room temperature. Once the reaction was completed, 2 mL of methanol was added at 0°C to destroy the excess of NaH. The reaction mixture was extracted with 3x50 mL of ethyl ether, and the combined ether layers dried over sodium sulfate. After solvent removal, the residue of (11.9 g, Y=61%) was combined with 60 mL of 10% acetic acid, then heated (oil bath, 100°C), until the original emulsion disappeared (about 0.5 h).

After concentration, the residue was distilled in vacuo, giving 8.1 g (b.p. 116-118°C/50μ) (Y=89%).

Hexadecanyl Isopropylidene Glyceryl Ether, Scheme II Compound 2, Alternative

5 As another alternative procedure, to prepare Scheme II, Compound 2, NaH (0.6 g, 0.025 mole) was added to 4 mL of THF containing 1.3 g (0.01 mole) of isopropylidene glycerol at 0°C. Gas was generated. After ~0.5 h, 3.0 g (0.01 mole) of hexadecanyl bromide was added dropwise. The reaction mixture was stirred for 2 h at room temperature. Methanol was added at 0°C to destroy excess sodium hydride. The mixture was filtered, and the filtrate
10 evaporated. The residue was extracted with 2x60 mL ethyl ether. The extract was washed with brine and water and dried over Na₂SO₄. After concentration, 2.36 g of crude product was obtained (Y=59%). NMR: ¹H: δ (ppm) 4.24 (m, 1H, CH), 4.04-3.70 (qxd, 2H, CH₂), 3.44 (m, 4H, CH₂OCH₂), 1.40 (q, 2H, OCH₂CH₂, ³J_{HH}=7Hz), 1.33 (d, 6H, CCH₃), 1.22 (s, 28H, CH₂), 0.85 (t, 3H, CH₃, ³J_{HH}=7Hz).

15

Hexadecanyl Glyceryl Ether, Scheme II Compound 3, Alternative

Hexadecanyl isopropylidene glyceryl ether (150 mg, 0.4 mmol), 4 mg of TsOH·H₂O and 20 mL of methanol were stirred at 0°C overnight. Removal of the solvent gave 110 mg of a white solid, which was dissolved in 1 mL of chloroform, filtered and concentrated, and dried
20 (vacuum oven) leaving 105 mg of product (m.p. 54-56°C). Pure product was obtained by recrystallization from ether, m.p. 62-64°C. NMR: ¹H: δ (ppm) 4.93 (m, 1H, CH), 3.83-3.66 (qxd, 2H, CH₂OH), 3.49-3.41 (m, 4H, CH₂OCH₂), 2.80 (s, b, 2H, OH), 1.54 (m, 2H, CH₂CH₃), 1.23 (s, 26H, CH₂), 0.85 (t, 3H, ³J_{HH} = 7Hz, CH₃); ¹³C: δ (ppm) 72.4 (CHOH), 71.8, 70.5 (CH₂OCH₂), 64.0 (CH₂OH), 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28.9.

22.7 (CH₂), 14.1 (CH₃); HRMS: 317.3047 (Found), 317.4056 (Calcd); Microanalysis: C%: 72.12 (Found), 72.10 (Calcd), H% 13.05 (Found), 12.74 (Calcd).

Synthesis of Scheme II Compound (5)

5 The Scheme II compound (4) (0.75 g, 1.3 mmole), 3 mL of pyridine, and 1 mL of acetic anhydride were stirred for 45 h at room temperature. 2 g of ice was added to the reaction mixture. 1.5 mL of chloroform was added to the mixture. The separated oil layer was then washed with 10% NaHCO₃ and water. The oil layer was rotary-evaporated after drying over MgSO₄. The residue was co-evaporated with 15 mL of toluene. 0.72 g of crude
10 product was obtained. Purification was carried out by flash column chromatography, petroleum and ether, 7:3 as eluting agent giving 0.53 g (Y = 67%) of pure compound. After standing, the product solidified, m.p. 43-44°C, NMR: ¹H: δ (ppm) 7.40-7.18 (m, 15H, Ph-H), 5.15 (m, 1H, CH), 3.62-3.16 (m, 6H, OCH₂), 2.05(s, 3H, COCH₃), 1.48 -1.21 (m, 28H, CH₂), 0.84 (t, 3H, ³J_{HH} = 7.0Hz CH₃); ¹³C: δ (ppm) 170.5(C=O), 143.8, 129.0, 128.6, 127.2(Ph-C),
15 86.5 (cph₃), 71.5 (CH), 69.4, 62.5, 61.0(OCH₂), 31.9, 29.7, 26.0, 22.7 (m, CH₂), 21.8(CH₃CO), 14.10 (CH₃), Analysis: C%: 79.96 (Found), 80.40 (Calcd), H% 9.39 (Found), 9.52 (Calcd).

Synthesis of Scheme II Compound (6)

20 The Scheme II compound (5) was dissolved into 2 mL of CH₂Cl₂ and 0.6 mL of 50% BF₃·MeOH was added at about 0°C to room temperature, while stirred for 4 hr. After concentration, the residue was purified by preparative TLC (petroleum and ether (7+3) as eluting agent) to give 54 mg of product (R_f = 0.28) (waxy material). NMR: ¹H: δ (ppm) 5.05 (m, 1H, CH), 3.62-3.16 (m, 4H, OCH₂), 2.05 (b, t, 1H, CH₂OH), 1.48-1.21 (m, 28H, CH₂),

0.86 (t, 3H, $^3J_{\text{HH}}=6.9\text{Hz}$, CH₃), ^{13}C : δ (ppm) 171.1 (CO), 71.75(CH), 71.3, 68.8, (OCH₂), 65.6(CH₂OH), 31.9, 29.7, 26.0 22.7(m, CH₂), 21.2(CH₃CO), 14.2 (CH₃).

Scheme II Compound 7 and 8

- 5 Using the hexadecan-1-yl isopropylidene ether prepared above one conducts the steps shown in Scheme II to prepare, in succession, compounds 7 and 8 of Scheme II. Thus, 50.8 mg (0.15 mM) of scheme II compound 6 in 4 ml of THF containing 15 mg of triethylamine and about 1 equivalent of either CIPFA or CLTPFA was stirred at 0°C for 2 h. After filtration and rotary evaporation of the solvent, the residue, Scheme II compound (7) was
- 10 purified on silica gel (CH₂Cl₂: CH₃OH, 95:5): ^{31}P NMR: δ -3.6. Scheme II Compound 7 was then hydrolyzed to Scheme II Compound 8 by methods known by those skilled in the art. The preparation of methyl chloro(methoxy)thiophosphonoformate (CITPFA) was as follows: Lawesson's reagent (10.7 g, 97%, 0.025 mole) was dissolved in 100 ml of dry toluene at 105°C followed by 4.0 g (0.02 mole) methyl chloro(methoxy)phosphonoformate (CIPFA)
- 15 [prepared by the method of Petrov, K. A., Maklyaev, F. L. and Korshunov, M. A. J. Gen. Chem. USSR (Eng. Transl.) 29, 304-308 (1959)]. After 18 hr at reflux, the solvent was removed in vacuum and the residue extracted with hexane. The combined extracts were evaporated leaving the crude product, which was distilled in vacuo to give 0.86 g of a slightly yellow oil, b.p. 40-40.5 °C at 0.075 mm Hg. ^1H -NMR (CDCl₃): δ 3.97 (d, POCH₃, 3H,
- 20 $^3J_{\text{PH}}=14$), 3.94 (s, COCH₃, 3H). ^{13}C -NMR (CDCl₃): δ 164.9 (d, CO, $^1J_{\text{PC}}=224$), 54.7 (d, POCH₃, $^2J_{\text{PC}}=8.5$) 54.5 (d, COCH₃, $^3J_{\text{PC}}=4.9$). ^{31}P NMR (CDCl₃): δ 62.4. Calcd for C₃H₆O₃ClPS: C, 19.11; H, 3.21; S, 18.80. Found: C, 19.42, H, 3.51, S, 18.80.

EXAMPLE 18

Scheme III Compound (2)' and (4)'

To a suspension of isopropylidene glycerol (760 mg, 5.214 mmol) and NaH (240 mg, 5.88 mmole) in THF were added tetrabutyl ammonium bromide (168mg, 0.52 mmol) at room temperature under N₂ followed by 4-methoxybenzyl chloride (921 mg, 5.88 mmol). After being refluxed at 65°C for 14 h, the reaction was quenched with methanol (1mL) and neutrallized with NH₄Cl. The reaction mixture was extracted with ether and the extract was washed with brine, dried over MgSO₄, and concentrated in vacuo. The residue was purified with flash chromatography on silica gel with ether and hexane, 1:1. 1.54 g of yellowish oil, with a yield of about 88.6%, was obtained. NMR: ¹H: δ (ppm) 7.27, 7.25 (dd, 4H, Ph-H), 4.51 (m, 1H, CH), 4.14 (d, 2H, OCH₂), 3.96 (m, 4H, OCH₂), 3.80 (s, 2H, PhCH₂), 1.42 (s, 3H, CH₃), 1.36 (s, 3H, CH₃), ¹³C: δ (ppm) 129.3, 130.1, 130.0, 114.1, 113.8 (Ph-C), 109.3 (C(CH₃)₂), 74.7 (CH), 73.1, 70.7, 66.9 (OCH₂), 55.1 (OCH₃), 26.75, 25.3 (CH₃). HRMS: 252.1371 (Found), 252.1361 (Calcd), Analysis: C%: 66.85 (Found) 66.65 (Calcd), H%: 7.58 (Found), 7.99 (Calcd). 3-p-methoxybenzyl-1,2-isopropylidene glyceryl ether, Scheme III compound (2)' was deprotected in 10 mL 10% acetic acid at 65°C for 1hr. The reaction mixture was lyophilized and the residue was purified by flash column chromatography, ethyl acetate as eluting agent. NMR: ¹H, ¹³C consistent with structure.

20

Scheme III compounds (3)', (5)' and (6)'

The R group was hexadecanyl. Scheme III compound (4)' (1.15 g 4.7 mmol), powdered KOH (1.06 g, 19 mmol), hexadecyl bromide (5.73 g, 20 mmole) and 25 mL toluene were mixed. The reaction mixture was refluxed for 16 hr at room temp. Next, 20 mL of water was added and the oil layer separated. The aqueous layer was washed by toluene and the combined oil phases washed with water brine. After drying over Na₂SO₄ and

concentrating. 7.1 g of crude product, Scheme III Compound (3)', was obtained. This was purified by flash chromatography (using hexane and ethyl ether, 9:1). $R_f = 0.44$. NMR: ^1H , ^{13}C consistent with structure.

To a mixture of Scheme III Compound (3)' (1.0 g, 1.5 mmol) in CH_2Cl_2 (9 mL) and
5 H_2O (0.5 mL) was added DDQ (2,3-dichloro-5,6-dicyanobenzoquinone, 0.36 g, 1.58 mmol). The reaction mixture turned progressively red-brown. After 15 min at room temperature the reaction mixture was filtered, and the filtrate was evaporated in vacuo giving a yellowish solid (0.75 g). This was purified by flash column chromatography (using hexane and ether, 3:1) to give Scheme III Compound (5)', m.p. 50-51°C (Y=93.8%) NMR: ^1H : δ (ppm) 3.68
10 (m, 1H, CH), 3.60-3.39 (m, 8H, OCH_2), 2.16-2.13 (dd, 1H, CH_2OH), 1.31-1.24 (m, 56H, CH_2), 0.86 (t, 6H, $^3J_{\text{HH}}=6.9\text{Hz}$); ^{13}C : δ (ppm), HRMS: 540.5568 (Found), 540.5481 (Calcd), Analysis: C%: 77.39 (Found), 77.71 (Calcd), H%: 13.23 (Found), 13.42 (Calcd). Scheme III Compound (6)' (90 mg, 0.28mM) and 2 ml pyrimidine were dissolved in 1.5 ml chloroform together with 1 equivalent of CIPFA or CLTPFA, which was added at -10°C. The
15 temperature was allowed to rise to r.t., then the reaction mixture was filtered and the filtrate concentrated by rotary evaporation. The residue was purified by column chromatography on silica gel ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$, 95:5) giving Scheme III Compound (6)' with $R_f = 0.26$. ^{31}P NMR: δ -3.6. Scheme III Compound (7)' was demethylated by methods known to those skilled in the arts.

20 The following references, discussed above, are all incorporated herein by reference:
U.S. Patent No. 5,183,812 to McKenna; D.W. Hutchinson, et al., "Synthesis and Biochemical Properties of Some Pyrophosphate Analogues", Biophosphates and Their Analogues-
Synthesis, Structure, Metabolism and Activity, K.S. Bruzik and W.J. Stec (Eds.), Elsevier
Science Publishers, B.V., 1987, 441-450; Helgstrands, et al., *Science*, 201:819-821 (1978); J.
25 Levillain, et. al., *J. Am. Chem. Soc.* 115:8444-8446, 1993; L.V. Kovalenko, et al., *Russian J.*

- General Chemistry* 64, Part 1, 1456-1459, 1994); D.W. Grisley, Jr., *J. Org. Chem.* 26, 2544-2546, 1961; U.S. Patent No. 5,072,032 to C.E. McKenna, et al.; Irwin B. Douglass, *J. Am. Chem. Soc.* 78:6070 (1956); S. Masson et al., *Tetrahedron Lett.* 31 1151 (1990); Ryu, et al, *J. Med. Chem.* 25, 1322-1329 (1982); M. Fuji, et al., *J. Org. Chem.*, 62, 6804 (1997); K.
- 5 Hostetler, et al., *Antiviral Research*, 31, 59-67 (1996); and Dieter Martin and Wolfgang Mocke, *Chemische Berichte*, 98 (7), 2059 (1965).

The compounds of this invention are proposed for use in standard assays for HIV-1 reverse transcriptase. The procedures therefor that can be used, *mutatis mutandis*, and described in McKenna U.S. Patent No. 5,072,032, the disclosure of which is incorporated

10 herein by reference. They also are proposed for use for the treatment of herpes virus infections in AIDS patients, in medical uses such as antiviral compounds, and in agricultural uses such as pesticides.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that

15 various modifications and changes which are within the knowledge of those skilled in the art are considered to fall within the scope of the appended claims.

THE CLAIMS

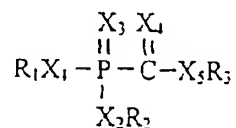
1. A thio-analog of phosphonoformic acid comprising the formula:



wherein R_1 and R_2 are each independently selected from alkyl, aryl, H, and cation, R_3 is independently selected from alkyl, aryl, H, cation, and a prodrug moiety, and X_1 , X_2 , X_3 , X_4 , and X_5 are each independently selected from O and S, provided that:

- 10 (a) at least one of $\text{X}_1 - \text{X}_5$ is S;
 (b) when X_1 is S, then either (i) R_1 or R_2 is alkyl, aryl, or H, or (ii) at least one of X_2 , X_3 , X_4 , and X_5 is also S.

2. A method of treating a human infected with the human immunodeficiency virus (HIV)
 15 to inhibit HIV activity comprising the administration of a pharmaceutically effective amount of a compound comprising the formula:

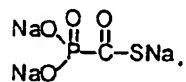


- wherein R_1 and R_2 are each independently selected from alkyl, aryl, H, and cation, R_3 is
 20 independently selected from alkyl, aryl, H, cation, and a prodrug moiety, and X_1 , X_2 , X_3 , X_4 , and X_5 are each independently selected from O and S, provided that:

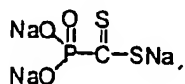
- (a) at least one of $\text{X}_1 - \text{X}_5$ is S; and
 (b) when X_1 is S, then either (i) R_1 or R_2 is alkyl, aryl, or H, or (ii) at least one of X_2 , X_3 , X_4 , and X_5 is also S.

25

3. The thio-analog of claim 1 comprising the formula:

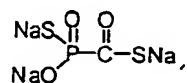


4. The thio-analog of claim 1 comprising the formula:

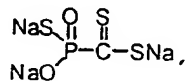


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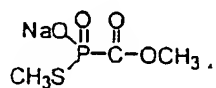
5. The thio-analog of claim 1 comprising the formula:



10 6. The thio-analog of claim 1 comprising the formula:

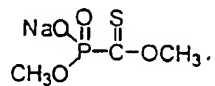


7. The thio-analog of claim 1 comprising the formula:



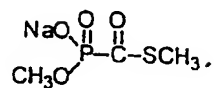
15

8. The thio-analog of claim 1 comprising the formula:

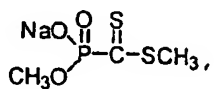


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9. The thio-analog of claim 1 comprising the formula:

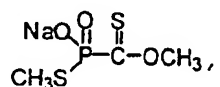


10. The thio-analog of claim 1 comprising the formula:

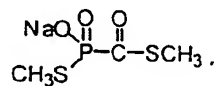


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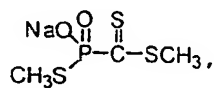
11. The thio-analog of claim 1 comprising the formula:



10 12. The thio-analog of claim 1 comprising the formula:

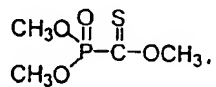


13. The thio-analog of claim 1 comprising the formula:

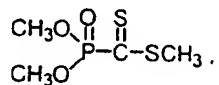


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14. The thio-analog of claim 1 comprising the formula:

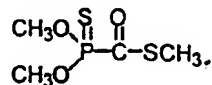


15. The thio-analog of claim 1 comprising the formula:

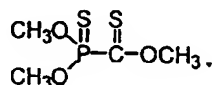


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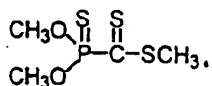
16. The thio-analog of claim 1 comprising the formula:



5 17. The thio-analog of claim 1 comprising the formula:

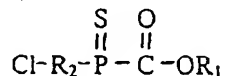


18. The thio-analog of claim 1 comprising the formula:



10

19. A composition comprising the formula:



wherein R_1 and R_2 are an alkyl or aryl group.

15

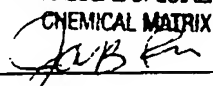
20. The composition of claim 19 wherein said alkyl group is methyl.

21. A process for the preparation of methyl chloro(methoxy)thiophosphonoformate comprising:

- 20 (a) forming a reaction mixture of an alkyl chloro(alkoxy)phosphonoformate with an excess amount of Lawesson's reagent; and
- (b) maintaining said mixture at reflux until said reagent is consumed.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/15775

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) :A61K 31/66; C07F 9/40 US CL :514/120; 558/181 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/120; 558/181 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN® International, REGISTRY Database, based on molecular structure		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3,943,201 A (MCINTOSH) 09 March 1976, col. 1, line 25, through col. 2, line 10.	1, 9, 10
X	US T986,003 H (BARRIER) 04 September 1979, see the abstract.	1
X	US 5,183,812 A (MCKENNA) 02 February 1993, see columns 15 through 17.	1, 2
X	WUTZLER, P. Therapeutic effect of pyrophosphate analogues on cutaneous herpes simplex virus type 1 infection in guinea pigs. Antiviral Research. 1988, Vol. 10, pages 99-106, especially page 101, Figure 1.	1
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "A" document member of the same patent family	
Date of the actual completion of the international search 28 SEPTEMBER 1999		Date of mailing of the international search report 07 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer MICHAEL G. AMBROSE Telephone No. (703) 308-1235 JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX 

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/15775

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database CAPLUS on STN International ^(SM) , Chemical Abstracts Service, (Columbus, Ohio), Accession No. 1993:168; STRAW, J.A. et al. 'Pharmacokinetics of potential anti-AIDS agents thiofoscarnet and foscarnet in the cat'. J. Acquired Immune Defic. Syndr. 1992, Vol. 5, No. 9, pages 936-942, abstract.	1,2
X	Database CAPLUS on STN International ^(SM) , Chemical Abstracts Service, (Columbus, Ohio), Accession No. 1995:400772; KOVALENKO, L.V. et al. 'Synthesis of dialkoxyposphorylthioformic acid amides'. Zh, Obshch. Khim. 1994, Vol. 64, No. 10, pages 1639-1641, abstract.	1, 14
X	Database CAPLUS on STN International ^(SM) , Chemical Abstracts Service, (Columbus, Ohio), Accession No. 1995:400771; KOVALENKO, L.V. et al. 'Synthesis of phosphonothioformate esters'. Zh, Obshch. Khim. 1994, Vol. 64, No. 10, pages 1634-1638, abstract.	1, 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/15775

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-18

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/15775

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-18, drawn to phosphinothiylformic acid derivatives.

Group II, claim(s) 19 and 20, drawn to chloroalkyl- or chloroarylphosphinothiylformate derivatives.

Group III, claim 21, drawn to a process for making chloroalkyl- or chloroaryl(methoxy)phosphinothiylformate derivatives.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The compounds of Group I represent derivatives of thio-analogs of phosphonoformic acid (two O-P or S-P bonds in addition to P-S and one C-P bond). The compounds of Group II as claimed are derived from phosphinic acid (i.e., at least two C-P bonds are present versus one in the Group I compounds); in fact the structure shown in claim 19 appears to be incomplete because the phosphorus atom is shown as tetravalent instead of as pentavalent. The process of Group III relates to the preparation of an alkyl chloro(methoxy)phosphonoformate (i.e., Cl-P bond). The compounds of group II comprise a *chlorine-carbon* bond; the chlorine atom being indirectly bonded to phosphorus instead of directly bonded as in group III. The groups of inventions outlined above include three different classes or organophosphorus compounds wherein the phosphorus atom is bonded to differing adjacent atoms.